Comments on the draft NTC Technical Report on the Toxicology and Carcinogenesis studies of isoeugenol (CAS No: 97-54-1) in F344/N Rats and B6C3F1 Mice (Gavage Studies). Peer Review Date: February 27-28, 2008

Genotoxicity aspects

The draft "NTP Technical Report on the Toxicology and Carcinogenesis studies of Isoeugenol in F344/N Rats and B6C3F1 Mice" contains results from 2-year gavage studies in rodents and genotoxicity studies. Whilst the conclusions made in the report refer only to the results of the rodent 2-year studies it is noted that the evaluation guidelines given on page 11 of the report make provision for consideration of other key factors, including the results of genotoxicity evaluations, that may extend the boundary of categories of evidence of carcinogenic potential based on statistical comparison of observed tumour incidences by incorporation of scientific experience and current understanding of long-term carcinogenesis studies in animals.

Genotoxicity reports are presented in the draft NTP toxicity report on isoeugenol. ScanAqua, Norway, recently commissioned a study of the potential for orally administered isoeugenol to induce micronuclei in the bone marrow of mice according to OECD guideline "Mammalian Erythrocyte Micronucleus test, No 474". According to this report it was concluded that isoeugenol did not show any in vivo genotoxic activity in this test system. A copy of the report was made available to NIEHS in 2007. The overall evidence regarding the genotoxic potential of isoeugenol is therefore as summarised below:

Genotoxicity studies presented in the draft NTP Technical Report (Appendix E):

Salmonella typhimurium, mutagenicity test protocol of NTP (in draft review):

Negative results.

Chinese hamster ovary cell cytogenetics protocols of NTP (in draft review):

Negative results.

Mouse peripheral blood micronucleus test protocol of NTP (in draft review):

Male mice: Negative results
Female mice: Positive at highest dose (600 mg/kg)

Note that the difference between control male mice and control female mice is proportionally greater than the difference between treated male and female mice. Data on historical controls or comparison with data of known carcinogens are not presented.

New study: Mouse micronucleus test (ScanAqua/LAB Research) (OECD 474):

Male mice: Negative
Female mice: Not tested

Positive control (Cyclophosphamide) approx. 25-30 times higher number of micronucleated PCE:s than in vehicle control and in isoeugenol test groups.

A summary of this report is enclosed.

Request to be considered

The weight of evidence clearly suggests that isoeugenol can be regarded as a non-genotoxic compound and it is requested that consideration be given to the insertion of a comment in the conclusion of the NTP Technical Report stating that there is no strong evidence to support a genotoxic mechanism of action for the statistically significant increase in the incidence of hepato-cellular tumours observed in male mice.

On behalf of the Sponsor

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Isoeugenol, mouse micronucleus test

Summary

The study was performed at LAB Research (Scantox), Lille Skensved, Denmark sponsored by ScanAqua AS, Aarnes, Norway.

The objective of the study was to determine whether isoeugenol caused genotoxic effects resulting in the formation of micronuclei in erythrocytes of treated mice. The test was conducted in accordance with the OECD guideline No. 474 (1997) “Mammalian Erythrocyte Micronucleus Test. The study was carried out in compliance to the OECD Principles of Good Laboratory Practice. Isoeugenol was dissolved in corn oil and dosed by gavage at 500, 1000 and 2000 mg/kg body weight. The vehicle, corn oil, was used as negative control. Cyclophosphamide, 20 mg/kg body weight was used as positive control. The test animals were of the strain Bom Tac:NMRI. In the main test 5 male mice per test group were used. The mice were sacrificed 24 hours after dosing. One additional control group and one additional highest dose group were sacrificed after 48 hours. Slides with Giemsa stained bone marrow preparations were prepared from the femurs. The slides were coded to assure unbiased examination.

Summary of results and statistical analysis

Sacrifice time (h)	Group	Treatment	MnPCE Range	Mean		%PCE Mean
24	1	Vehicle control	1 - 3	2.0		47.6
24	2	Isoeugenol (500 mg/kg)	2 - 3	2.4	ns	45.6
24	3	Isoeugenol (1000 mg/kg)	1 - 4	2.2	ns	39.4
24	4	Isoeugenol (2000 mg/kg)	1 - 3	2.2	ns	39.3
24	5	Cyclophosphamide (20 mg/kg)	47 - 69	59.0	**	37.5
48	1	Vehicle control	1 - 3	2.2		45.8
48	4	Isoeugenol (2000 mg/kg)	1 - 2	1.8	ns	39.5

MnPCE	Number of polychromatic erythrocytes (PCE) with micronuclei (2000 PCE scored/animal)
%PCE	Frequency of PCE among total erythrocytes (%) (1000 erythrocytes scored/animal)
ns	Difference from vehicle control not statistically significant at 5% level ($p>0.05$)
*	Statistically significant difference from vehicle control at 5% level ($0.05>p>0.01$)
**	Statistically significant difference from vehicle control at 1% level ($p<0.01$)

Conclusion

Isoeugenol did not show any genotoxic activity in this mouse micronucleus test.

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